Abstract: This study investigated the difference in neonicotinoids dissipation in a grape vineyard by planting different groundcovers plants, including a control bare field (CF), *Arachis pintoi* Krap. and Greg. (peanut field (PF)) and *Clinopodium brownei* (Sw.) Kuntze (mint field (MF)). After one day of pesticide spraying, the highest dinotefuran residue concentration was in 0- to 15-cm soil in the CF (0.161 mg/kg), but 30- to 45-cm and 15- to 30-cm soil in the MF and PF, respectively (0.307 and 0.033 mg/kg). Also, after four days, the highest imidacloprid residue concentration was in 0- to 15-cm soil in the CF. Imidacloprid was not retained in the 30- to 45-cm soils in the PF, but in the MF, a 0.015- and 0.011-mg/kg residue was detected in 30- to 45-cm soil in the second and third soil samplings, indicating a different distribution with different groundcover plants. The dinotefuran absorption ability was greater with *A. pintoi* than *C. brownei*, and the imidacloprid absorption ability was greater with *C. brownei*. Our results suggest that groundcover plants affect the dissipation of neonicotinoids differently, while *A. pintoi* has a high metabolic rate toward the two neonicotinoids and can increase the soil organic matter content, which is a preferable choice for a groundcover.

Keywords: ground cover plants; vineyard; dinotefuran; imidacloprid

1. Introduction

Orchard-floor management methods include retaining specific types of weeds in a strip or placing manure and planting non-native grass on the orchard floor. The primary objectives are to suppress weeds, but also to reduce herbicide use while improving the soil’s physical, chemical, and biological functions. Recently, planting groundcover has been included among the environmental-friendly strategies of orchard-floor management [1].

In Taiwan, we apply and promote groundcover planting especially in deep-rooted orchards, usually in mountainous regions. This management type can increase the soil’s organic carbon and nutrient levels, including nitrogen, exchangeable potassium, calcium, and magnesium, although the nutrition levels in the crop may not be significantly increased [2,3]. The technique can modify the soil’s physical properties, including the water infiltration rate and macropore content [4,5]. The changes in soil characteristics with groundcover planting may greatly affect the distribution of pesticides, especially high water-soluble pesticides such as neonicotinoid insecticides. However, we have little knowledge of this effect.

Neonicotinoid insecticides are systemic insecticides that, regardless of their route of entry, can be distributed throughout the plant and harm feeding insects [6]. They are applied by seed treatment,
foliar sprays, soil drenches, granules, and injection or irrigation systems [7]. The sales of imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, and dinotefuran are the highest among the neonicotinoids in the United States [8]. The global market share of neonicotinoids was greater than 25% in 2014; in 2012, thiamethoxam, imidacloprid, and clothianidin accounted for almost 85% of the total neonicotinoid sales for crop protection [9]. In Taiwan, imidacloprid, acetamiprid, and dinotefuran are promoted to farmers to prevent and control thrips in vineyards [10].

Neonicotinoids are quickly dissipated in soil [11]. The half-life of dinotefuran is 16.5 to 21.7 days [12], whereas with imidacloprid, only 130 days is required for 652 µg/kg to dissipate to 11 µg/kg in the field [13]. Nevertheless, long-term accumulation and persistence in water and soil samples were reported. Levels higher than 0.1 µg/kg of imidacloprid were detected in soils not planted with imidacloprid-coated seeds for one year [11]. Neonicotinoids are potential groundwater contaminants; the sorption coefficient (Koc) of dinotefuran is 30, whereas that of imidacloprid is 262, and the water solubility is 39,800 and 514 mg/L, respectively [14,15]. Since the mid-2000s, studies raised concerns that neonicotinoids may have a negative effect on the non-target organisms, such as honeybees and bumblebees. The European Food Safety Authority (EFSA) assessed the risk of clothianidin, imidacloprid, and thiamethoxam, and concluded that the application of these compound poses a high risk to bees [16]. Planting groundcover could reduce the environmental risk of neonicotinoids more than hydrophobic pesticides.

This study aimed to investigate the change in distribution of neonicotinoids in a groundcover-planted grape vineyard. The groundcover plants were *Arachis pintoi* Krap. and Greg. and *Clinopodium brownei* (Sw.) Kuntze, two intensively promoted and cultivated groundcovers in Taiwan, to enhance soil properties in the vineyard. *Arachis pintoi* is a legume groundcover [3], whereas *Clinopodium brownei* (Sw.) Kuntze was recently promoted for its potential as an insect repellent with a unique peppermint smell. We hoped to obtain in-depth knowledge about the effect of groundcover planting on the dissipation of the neonicotinoids dinotefuran and imidacloprid in the vineyard, and thus provide a reference for policy-making and groundcover management in vineyards.

2. Materials and Methods

2.1. Vineyard, Groundcover Plants, and Experimental Design

The grape vineyard (*Vitis vinifera* L. × *Vitis labruscana* Bailey cv. Kyoho) was located in the Taichung District Agricultural Research and Extension Station, Chunghua, Taiwan (24°00′07.5″N 120°32′04.6″E), with a subtropical climate, temperature averaging 23 °C, and an average rainfall of 1488 mm. The groundcover plants chosen were among those promoted by the institute to enhance soil properties in the vineyard, namely: *A. pintoi* Krap. and Greg. (peanut field [PF]) and *C. brownei* (Sw.) Kuntze (mint field [MF]). The experiment was conducted in three treatment fields in randomized complete block design (RCBD) design, namely: MF, PF, and a bare control field (CF). Each treatment was done in three replicates, so the vineyard was randomly divided into nine plots (3*17 m²) with a 45-cm ditch at the edge of each plot to avoid plant invasion. The groundcover plants were grown from cuttings placed into the plots six months before the experiment, until they fully covered the designated plots. Before the experiment, the vineyard had been established for at least five years, following the guidelines from Taiwan Good Agriculture Practice [17], so the fertilizers and pesticides (including dinotefuran and imidacloprid) were applied according to government recommendations (see below) [10].

Before the experiments, three replicates of soil samples at a 0–15, 15–30, and 30–45 cm depth and groundcover plant samples were taken from each plot. The soils were examined for background values of pH [18], electrical conductivity (EC) [19], clay content [20], soil organic matter (SOM) content [21], and dinotefuran and imidacloprid levels before and after treatments. The surface soils were also examined for background concentrations of total nitrogen, available phosphorus, and exchangeable potassium by the soil survey and testing center at National Chung Hsing University, Taichung,
Taiwan. The groundcover plants were examined for dinotefuran and imidacloprid content before and after treatments.

2.2. Pesticide Application

Dinotefuran and imidacloprid were applied following the field-recommended doses and methods in the Taiwan Plant Protection Manual in three treatment fields [10]. For each hectare, dinotefuran was applied at 0.3 kg of 20% water soluble granule, and imidacloprid was applied at 0.5 L of 9.6% soluble concentrate. The applications and sampling dates are in Table 1. During the experiment, dinotefuran was applied only once, and imidacloprid was applied every one or two weeks.

| Table 1. Sampling dates, pesticide treatments, and days after last pesticide application. |
|-----------------------------------------------|------------------|------------------|------------------|------------------|
| Sampling Dates                          | Dinotefuran | Imidacloprid | Dinotefuran | Imidacloprid |
| Background                             | 29 August    | 4 September   | 1 September | 1 September |
| First sampling                        | 5 September  | 1             | 7 September | 4             |
| Second sampling                       | 12 September | 8             | 12 September | 5             |
| Third sampling                        | 5 October   | 31            | 26 September | 9             |

2.3. Soil and Plant Pesticide Extraction

Pesticide extraction involved the QuEChERS method (quick, easy, cheap, effective, rugged, and safe) [22] with modifications. Amounts of 5 g of wet soil (water content was predetermined following the method of Gardner, and equivalent amounts of dry soil were calculated afterward) [23] or 2.5 g of air-dried groundcover plant samples were added into 50-mL falcon tubes. An amount of 5 mL dH$_2$O was added to soil and 10 mL dH$_2$O was added to the plant samples before vortexing for 1 min. Then, 10 mL of acetonitrile containing 1% formic acid was added to the soil, and 20 mL of this solution was added to the plant samples for vortexing for 1 min. An AOAC pouch (Agilent Tech.) was added in tubes for vortexing for 1 min. The tubes were centrifuged at 3500 rpm (1600 g) for 10 min. The supernatant was placed into a 15-mL falcon tube containing dSPE (Agilent Tech.), vortexed for 1 min, and centrifuged again at 3500 rpm (725 g) for 10 min. An amount of 2 mL supernatant was blow-dried by using a nitrogen gas blowing concentrator, then dissolved in a proper amount of acetonitrile to obtain a 1 mL extract. The extract was filtered with 0.45-μm nylon membrane. The pesticide residues were determined as follows.

2.4. Pesticide Analysis and Standard Curve

Analytical-grade standard pesticides of weight 0.01 g were placed into a 10-mL volumetric flask to obtain a 1000 mg/kg stock solution. The stock solution was diluted to 0.01, 0.03, 0.05, 0.08, 0.10, and 0.30 mg/kg as the working standards. The pesticide levels were analyzed by high-performance liquid chromatography (Hitachi 1110 pump and 1410 Detector) equipped with a C18 column (5 μm, 250-4, Merck), with a detection wavelength of 280 nm for dinotefuran and 260 nm for imidacloprid; the mobile phase for dinotefuran and imidacloprid was 2:3 and 1:3 of acetonitrile:0.01% H$_3$PO$_4$. The flow rate for both pesticides was 1 mL min$^{-1}$. The standard curves and recovery rates of the pesticides are in Tables S1 and S2 in Supplementary.

2.5. Statistical Analysis

The data were analyzed by one-way ANOVA with SAS v9.4 (SAS Institute Inc., Cary, NC, USA). The post-hoc analysis involved the Fisher’s least significant difference (LSD) test for significant difference between treatments. $p < 0.05$ was considered statistically significant.
3. Results

3.1. Change in Soil Properties

The soil total nitrogen (N), available phosphorus (P), and exchangeable potassium (K) in 0- to 15-cm soils in the field samples were examined for the background content after six months of groundcover planting (Table 2). From the results, no significant differences were observed among all of the treatments.

Table 2. Soil total nitrogen (N), available phosphorus (P), and exchangeable potassium (K) in 0- to 15-cm soils in field samples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total N (%)</th>
<th>Available P (mg/kg)</th>
<th>Exchangeable K (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>0.49 ± 0.07</td>
<td>493.00 ± 58.03 a</td>
<td>367.67 ± 53.82</td>
</tr>
<tr>
<td>MF</td>
<td>0.49 ± 0.04</td>
<td>561.67 ± 19.22 ab</td>
<td>385.33 ± 87.31</td>
</tr>
<tr>
<td>PF</td>
<td>0.51 ± 0.04</td>
<td>565.67 ± 11.59 b</td>
<td>400.67 ± 62.52</td>
</tr>
</tbody>
</table>

a CF—control field; PF—A. pintoi Krap. and Greg. field (peanut field); MF—C. brownei (Sw.) Kuntze (mint field).
b Field data are mean ± standard deviation (SD).

Figure 1 shows the variation in soil pH in the top-layer soil (0–15 cm) among all of the fields after six months of planting, as follows: 7.47, 6.69, and 6.97 for CF, MF, and PF, respectively. With the increasing soil depth, the pH increased. The planting of the two groundcovers significantly decreased the soil pH in the soil layers, except in the 15- to 30-cm soil in the PF.

Figure 1. Soil pH after six months of groundcover planting.

a. CF: control field; MF: C. brownei field (mint field); PF: A. pintoi field (peanut field).
b. Uppercase letters represent significant differences for different treatments in the same soil layer; lowercase letters represent significant differences for the same treatment in different soil layers.
c. Bars without letters indicate no significant differences shown.

Almost all of the treatments did not significantly differ in EC; for example, the EC in the top-layer soil for CF, MF, and PF was 1206, 1288, and 1178 µS/cm, respectively, (Figure 2). Except for the MF, for the two other soils, the EC significantly decreased in the 30- to 45-cm soil (852.3 µS/cm).
Almost all of the treatments did not significantly differ in EC; for example, the EC in the top-layer soil for CF, MF, and PF was 1206, 1288, and 1178 \( \mu \text{S/cm} \), respectively (Figure 2). Except for the MF, for the two other soils, the EC significantly decreased in the 30- to 45-cm soil (852.3 \( \mu \text{S/cm} \)).

![Figure 2. Soil electric conductivity after six months of groundcover planting. See Figure 1 for explanations.](image)

The soil clay content significantly increased with the depth, but was not significantly decreased by the groundcover planting (Figure 3). From the surface to the 30-cm soil, the CF soils had a 5% higher clay content than both the MF and PF soil, whereas in the 30- to 45-cm soil, the MF and PF soil had a 2% and 4% higher clay content than the CF soil.

![Figure 3. Clay content after six months of ground cover plant growing. See Figure 1 for explanations.](image)

The SOM content was the highest in the surface soils in all of the fields (Figure 4); the content was 7.58%, 8.22%, and 8.56% in the CF, MF, and PF soil, respectively. The increase in SOM content was most evident in the 15- to 30-cm PF soil, significantly higher by 1.69% than in the CF soil. However, the SOM content did not significantly differ in the CF and PF soils at 30–45 cm. The SOM content in the 15- to 30-cm soil was higher by 1.16% in the MF than the CF soil, but not significantly.
3.2. Dinotefuran Level in Soil and Plants

A trace amount of dinotefuran was detected in the soil and plants from the samples taken on 29 August, when no pesticides were being sprayed during the two months of the grape dormant period (Figure 5). Thus, all of the dinotefuran residues detected were from the previous growing season. In the background CF soil, 0.007 mg/kg dinotefuran was detected in the 30- to 45-cm soil (Figure 5a), whereas in the background MF soil, it was detected in the three soil layers (0.007, 0.020, and 0.006 mg/kg, respectively (Figure 5b)). No dinotefuran residue was found in the background PF soil before pesticide sprays (Figure 5c).
The dinotefuran level did not peak one day after dinotefuran spraying, perhaps because the pesticides were spayed mostly toward the grapevine and leaves, so a higher soil concentration of pesticides would be detected in the soils only after settling down from the air, and leaching from the plants, which took more than one day. The dinotefuran residue was detected 1, 8, and 31 days (first, second, and third samplings, respectively) after application. The dinotefuran level in the CF was the highest in the surface soil on day eight (0.161 mg/kg) (Figure 5a). However, in the MF (Figure 5b), the level was the highest in the 30- to 45-cm soil at day eight (0.307 mg/kg). In the PF (Figure 5c), the level peaked in 15- to 30-cm soil on day eight (0.033 mg/kg). Preserving the bare land in a vineyard would retain a high amount of dinotefuran in the surface soil right after its application, whereas A. pintoi planting seemed to have the lowest dinotefuran residue.

At 31 days, the dinotefuran level in the CF was the highest in the surface soil (0.035 mg/kg) (Figure 5a), but was highest in the MF and PF in 30- to 45-cm soil (0.039 mg/kg) and 15- to 30-cm soil (0.025 mg/kg), respectively (Figure 5b,c). C. brownei seemed to have a greater groundwater contamination potential than A. pintoi.

On 29 August, the dinotefuran level was 1.278 and 0.605 mg/kg in the MF and PF, respectively (Figure 5d, background), although the difference was not statistically significant. After one dinotefuran application on 4 September, the pesticide level after one day was 4.055 and 12.285 mg/kg in the MF and PF, respectively. After one month, the level dropped to 0.717 and 0.355 mg/kg, respectively; thus, A. pintoi showed a greater absorption and metabolic ability for dinotefuran as compared with C. brownei.

3.3. Imidacloprid Level in Soils and Plants

A trace amount of imidacloprid was detected in both the soils and plants from the samples taken on 29 August (Figure 6), when no pesticides were being sprayed during the two months of the grape dormant period. Thus, all of the imidacloprid residues detected were from the previous growing season.

![Figure 6. Imidacloprid level in soil and plants. (a) CF; (b) MF; (c) PF; (d) levels in plants. (1) 0–15 cm; (2) 15–30 cm; (3) 30–45 cm soil. BG—background; 1st—first sampling; 2nd—second sampling; 3rd—third sampling. See Figure 1 for explanations.](image-url)
In the background CF soil, 0.024 and 0.028 mg/kg imidacloprid was detected in the surface and the 15- to 30-cm soil (Figure 6a), whereas in the MF, imidacloprid was detected in the following three soil layers: 0.018, 0.040, and 0.017 mg/kg, respectively (Figure 6b). The imidacloprid level was the highest in the surface and the 15- to 30-cm soil in the PF, 0.064 and 0.044 mg/kg, respectively (Figure 6c).

The first sampling date after imidacloprid application was 5 September, four days after treatment. A high imidacloprid level remained in the surface soil in all of the fields, as follows: 0.084, 0.084, and 0.123 mg/kg in the CF, MF, and PF, respectively. However, for the CF and PF, in the second and third samplings, imidacloprid was detected only from the surface to the 30-cm soils, but not in the 30- to 45-cm soil. For the MF, 0.015 and 0.011 mg/kg imidacloprid was detected in the deep soil layer. Thus, a trace amount of imidacloprid in the 30- to 45-cm soil was found only in the *C. brownei* field after successive application of pesticides. Again, preserving the bare land in a vineyard would retain a high amount of imidacloprid in the surface soil, but planting *C. brownei* seemed to confer a high groundwater contamination potential as compared with planting *A. pintoi*.

The imidacloprid level with planting *C. brownei* and *A. pintoi* was 0.347 and 0 mg/kg in the grape dormant period (Figure 6d). After consecutive application of imidacloprid, in the first to third samplings, the levels were 0.868, 5.477, and 5.021 mg/kg with *C. brownei*, and 1.299, 1.871, and 1.078 mg/kg with *A. pintoi*, respectively. The absorption ability of imidacloprid seemed higher with *C. brownei* than *A. pintoi*, whereas the metabolic ability seemed higher with *A. pintoi* than *C. brownei*, because the imidacloprid residue could not be detected in the background.

### 4. Discussion

This study investigated the difference in neonicotinoid dissipation in a grape vineyard by planting two different groundcovers for orchard-floor management. After one day of pesticide spraying, the highest dinotefuran residue concentration was in the 0- to 15-cm soil in the CF, but was in lower levels in the MF and PF; after four days of pesticide application, the highest imidacloprid residue concentration was in the 0- to 30-cm soil in the CF. Imidacloprid was not retained in the 30- to 45-cm soils in the PF, but in the MF, residue was detected in 30- to 45-cm soil in the second and third samplings, which indicates a different distribution of neonicotinoids with different groundcover plants. The dinotefuran absorption was greater with *A. pintoi* than *C. brownei*, and the imidacloprid absorption was greater with *C. brownei*. *A. pintoi* may have a high metabolic rate toward the two neonicotinoids in the field, and can increase the SOM content in lower-layer soil, for a preferable choice as groundcover vegetation.

Pesticides can be retained in the top layer of soils throughout a growing season [24]. In our study, the phenomena could be observed in our bare land control, showing a high amount of dinotefuran retained in the surface soil right after application, and in the imidacloprid retained almost throughout the experiment. The different distribution of the two neonicotinoids in the bare land control could be explained by the different physical properties of the two pesticides, such as Koc and water solubility [14,15]. Dinotefuran has better water solubility than imidacloprid, which may explain the higher plant absorption and higher groundwater contamination potential with *C. brownei* planting.

The growing of groundcover plants altered the soil physical properties and the distribution and dissipation patterns of the two pesticides tested. The change in pH may affect the degradation rates of the neonicotinoids in the soils. In a study that USEPA found acceptable (MRID# 45640118), dinotefuran has a longer half-life in pH 8.8 than pH 6.5 and 6.6 soils [25]. In addition, the increase in soil pH led to a greater persistence of imidacloprid (pH 8.5 > pH 6.9 > pH 5.2) [26]. The above studies showed that a decreased soil pH would accelerate the degradation rates of the two neonicotinoid insecticides.

Growing plants can reduce soil pH [27]. The growth of the two groundcovers significantly lowered the soil pH, which suggested enhanced degradation rates by growing groundcover plants. However, this phenomenon was observed only in the top layer soils with dinotefuran application. In the 30- to 45-cm soil, the dinotefuran residue was higher in the MF than CF, whereas imidacloprid
was detected in only the MF in the second and third samplings. A contradiction occurred if considering only the lower pH observed.

The soil clay and SOM contents are related to the adsorption of pesticides in soils, whereas the soil clay content is the most relevant factor for polar pesticides [28,29]. Other research indicated that dissolved organic carbon competes with neonicotinoids for binding sites on soil organic carbon [30]. Also, increased soil organic carbon by the application of organic fertilizers and manure increases the persistence of neonicotinoids in the field [31]. However, in some research, the total degradation or biodegradation of neonicotinoids was the fastest in the soil with the highest organic carbon content [32]. In this study, groundcover planting decreased the soil clay content, but increased the SOM content, especially in the deep layer soil in the PF.

However, a trace amount of dinofuran could still be found after 31 days with the three treatments. Hence, the soil property changes caused by groundcover planting did not facilitate in the thorough degradation of the two neonicotinoids in this study. Groundcover planting facilitated the downward movement of the pesticides in some cases, such as imidacloprid in the MF (Figure 6b). A trace of amount of imidacloprid was found in the 30- to 45-cm soil in the last two samplings of the MF soil, whereas in the CF and PF, imidacloprid was detected only from the surface to the 30-cm layer.

The study also suggested that the dissipation patterns of the two neonicotinoids varied depending on the groundcover grown, with C. brownei seeming to confer a greater groundwater contamination potential. This suggestion might be explained in part by the soil property differences tested in this study. For example, we observed a relatively neutral pH change and lower SOM content in the 30- to 45-cm soil with C. brownei planting, which would favor the lower degradation rates of the the pesticides, as compared with A. pintoi planting. Yet, the higher neonicotinoid leaching potential with C. brownei planting could result from the higher root density of C. brownei than A. pintoi, which was not examined in this study. More studies are needed in order to verify the relation between plant physiological traits and the field distribution patterns of the two pesticides.

Moreover, the ability and patterns of uptake, translocation, and metabolism of neonicotinoids varies in plants [33]. A groundcover plant equipped with a high absorption and metabolic ability toward neonicotinoid could reduce the possibility of contaminated groundwater. In this study, A. pintoi showed a higher absorption for dinofuran, and C. brownei showed a higher absorption for imidacloprid. However, the background levels of the two pesticides were lower with A. pintoi planting, which suggests its higher metabolic ability for the two pesticides, although a lab test in a control environment should be conducted in order to further verify the finding.

From our observations, A. pintoi would be a preferable choice as groundcover vegetation in vineyards, because of the increased SOM content but reduced possibility of neonicotinoid contamination to the environment.

5. Conclusions

The growing of groundcover plants in an orchard is an alternative management method that is among one of the environmentally friendly strategies promoted. However, the distribution of water-soluble pesticides changes with the growing of different groundcover plants, with different pesticide absorptions and metabolic abilities. Preserving bare land in a vineyard would retain a high level of neonicotinoids in the surface soil right after pesticide application, which would decrease the groundwater contamination potential. Although the SOM was increased with groundcover planting, planting C. brownei facilitated the downward movement of neonicotinoids into lower-layer soils. A. pintoi had a higher metabolic rate toward neonicotinoids, and could increase the SOM content in lower-layer soils, for a preferable choice to be recommended to the farmers in vineyards as groundcover vegetation.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/11/3/798/s1, Table S1: Standard curves for dinofuran and imidacloprid., Table S2: Recovery rates of dinofuran and imidaclorid amendment.

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Conflicts of Interest: The authors declare no conflict of interest.

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